

Transesterification Optimization of Neem (*Azadirachta Indica*) Oil to Biodiesel and Its Emission Characterization

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Abstract

Azadirachta indica methyl esters, AIME, (biodiesel) were produced via a two-step process of esterification and transesterification of seed oil with methanol in the presence of catalyst. In the first step, acid catalyst (H_2SO_4) was used while in the second step, alkali catalyst (NaOH) was employed. Response Surface Methodology (RSM) was applied to optimize the transesterification process, and the effects of reaction temperature, catalyst amount, reaction time and methanol/oil molar ratio, and their reciprocal interactions were ascertained. A total of 30 experimental runs were designed by Central Composite Rotatable Design (CCRD) and carried out. A quadratic polynomial was obtained for predicting the transesterification process and the ANOVA test showed the model to be significant ($p < 0.05$). The validity of the predicted model was confirmed by carrying out three independent replicates experiments. The actual maximum AIME yield obtained was 85.13% (w/w) at the reaction temperature of 50 °C, catalyst amount of 0.7 (wt.%) and methanol/oil molar ratio of 3 (v/v) with a reaction time of 60 min. The fatty acid profile of the AIME revealed the dominant fatty acid was linoleic (61.28%). The fuel properties of the AIME were within the ASTM D6751 and DINEN 14214 specifications. The CO and NO emission concentration of blends decrease by 45% and 40%, respectively, compared to the conventional diesel fuel thus, help reduction in emissions that have harmful effect on the environment.

Keywords: biodiesel, optimization, response surface methodology, transesterification, *Azadirachta indica* oil

Introduction

Biodiesel, which is considered as an alternative of convectional diesel is gaining ground as a biodegradable, non-toxic and environment-friendly fuel to neat diesel (Knothe et al., 2007; Demirbas, 2008). It is produced through a chemical process known as “transesterification or alcoholysis” in which there is displacement of alcohol from an ester under acidic or basic catalytic conditions producing free glycerol and the fatty acid esters of the respective alcohol (Knothe et al., 2007). Biodiesel is derived from renewable feedstock like vegetable oils or animal fats. Both edible and non-edible oils have been successfully employed in biodiesel production. Due to the food versus fuel challenge, the use of non-edible oils for this purpose is preferable. Some recent examples of non-edible oils used for biodiesel production include *Azadirachta indica* (Muthu et al., 2010), *Jatropha curcas* (Tiwari et al., 2007), Karanja (Naik et al., 2008).

Azadirachta indica, commonly called Neem is widely found in West Africa and Asia. It is ubiquitous in Northern Nigeria and fairly found in Western Nigeria, where it is usually referred to as *Dogon Yaro*. Neem seed has been reported by Mongkholkhajornsilp et al. (2005) to contain about 45% oil, which composes of polyunsaturated fatty acids: oleic (50–60%) and linoleic acid (8–16%) and saturated fatty acids: palmitic acid (13–15%), stearic acid (14–19%) and arachidic acid (1–3%). Fatty acid profile of Neem seed oil has been shown to vary from tree to tree as a result of the genetic variability (Singh et al., 1999). Muthu et al. (2010) converted the seed oil into biodiesel in a two-step transesterification process by using a solid acid catalyst (sulfated zirconia) and alkali catalyst (KOH) for the first and second steps, respectively. The authors optimized the process but without proper experimental design and statistical approach.

Response surface methodology (RSM) is a useful statistical tool, which has been applied in research for optimizing various processes including transesterification reaction of vegetable oils:

Moringa oleifera (Rashid et al., 2011), *Jatropha* oil (Tiwari et al., 2007) and cottonseed oil (Zhang et al., 2011). The main advantage of RSM is the ability to reduce number of experimental runs needed to provide sufficient information for statistically acceptable results. In this present study, an effort was made to optimize the process conditions for the transesterification step of *Azadirachta indica* seed oil using RSM.

Methodology

Extraction of Azadirachta indica seed oil

Azadirachta indica seeds were collected from Kano State, Nigeria. Chaff was separated from the oilseeds by winnowing. The cleaned oilseeds were milled into powder by grinding with plate machine. A 5-liter Soxhlet apparatus and ethanol as solvent were used for the oil extraction.

Experimental design of AIME production

In this study, the central composite rotatable design (CCRD) was employed to optimize the AIME production. Five-level-four-factors design was applied, which generated 30 experimental runs. This included 16 factorial points, 8 axial points, and 6 central points to provide information regarding the interior of the experimental region, making it possible to evaluate the curvature effect. Selected factors for the transesterification process from the *Azadirachta indica* seed oil were reaction temperature (X_1), catalyst amount (X_2), reaction time (X_3) and methanol/oil molar ratio (X_4). The coded levels of the independent factors are given in Table 1. The experiments were randomized to minimize the effects of unexplained variability in the observed response due to extraneous factors.

Table 1: Coding of Experimental Factors and Levels

Variable	Symbol	range and their levels				
		-2	-1	0	1	2
Reaction temperature (°C)	X ₁	50	52	54	58	60
Catalyst amount (wt %)	X ₂	0.7	0.8	0.9	1.0	1.2
Reaction time (min)	X ₃	50	52	54	58	60
Methanol/oil ratio	X ₄	3	4	5	5.5	6

Experimental procedure

Two-step transesterification reaction was applied for the AIME production, due to the high FFA value of the seed oil. Hence, the modified method of Hanny and Shizuko (2008) was employed. In the first step, 1% (w/w) of H₂SO₄ was added to 0.60% (w/w) methanol; the mixture was heated in a water bath at a 50 °C for 1 h. The mixture was added to a known weight of preheated seed oil in a glass reactor in order to reduce the FFA to <1.50. In the second step, a known weight of NaOH pellet was dissolved in a known volume of anhydrous methanol and was quickly transferred into the esterified seed oil in the reactor and the reaction was monitored according to the design variables. At the completion of the reaction, the product was transferred to a separating funnel for glycerol and AIME separation. Glycerol was tapped off and the AIME left was washed with distilled water to remove residual catalyst, glycerol, methanol and soap. The washed AIME was further dried over heated CaCl₂ powder. The AIME yield was determined gravimetrically as described in Eq.1

$$\text{Biodiesel Yield \% (w/w)} = \frac{\text{Weight of biodiesel produced}}{\text{Weight of Azadirachta indica seed oil used}} \quad (1)$$

Statistical Data Analysis

AIME production data was analyzed statistically using RSM, so as to fit the quadratic polynomial equation generated by the Design-Expert software version 8.0.3.1 (Stat-Ease Inc., Minneapolis, USA). To correlate the response variable to the independent factors, multiple regressions was used to fit the coefficient of the polynomial model of the response. The quality of the fit of the model was evaluated using test of significance and analysis of variance (ANOVA). The fitted quadratic response model is given by Eq. 2.

$$Y = b_0 + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_{ii} X_i^2 + \sum_{i < j}^k b_{ij} X_i X_j + e \quad (2)$$

Where, Y is response factor (AIME), b_0 is the intercept value, b_i ($i = 1, 2, \dots, k$) is the first order model coefficient, b_{ij} is the interaction effect, and b_{ii} represents the quadratic coefficients of X_i , and e is the random error.

Oil and fuel properties

Fuel properties namely, moisture content, specific gravity, kinematic viscosity at 40 °C, iodine value, acid value, saponification value, higher heating value, flash point, cloud point and cetane number of both *Azadirachta indica* seed oil and AIME were determined following standard methods and compared with American and European standards (ASTM and DIN EN14214).

Results and Discussion

Properties of the extracted Azadirachta indica seed oil

The analysis of the oil showed that it has a moisture content of 0.15%, specific gravity of 0.916 and kinematic viscosity of 13.80 mm²/s. The acid value of the oil was 10.21 mgKOH/g oil while

the iodine value was 78 g I₂/100g oil. Whereas the saponification value of the oil was 201.21 mg KOH/g oil, its higher heating value and cetane number were 40.01 MJ/kg and 54.38, respectively. These results are within the ranges earlier reported in the literature (Soetaredjo et al., 2008; Muthu et al., 2010).

Optimization of the transesterification step

Table 2 depicts the coded factors considered in this study with experimental results, predicted values as well as the residual values obtained. The highest AIME yield obtained was 89.69 % (w/w) at reaction temperature 54 °C, catalyst amount 0.90% (w/w), reaction time 50 min and methanol/oil molar ratio 5:1, while the lowest AIME yield of 60.50% (w/w) was observed at reaction temperature 54 °C, catalyst amount 0.70% (w/w), reaction time 54 min and methanol/oil molar ratio 5:1. Design Expert 8.0.3.1 software was employed to evaluate and determine the coefficients of the full regression model equation and their statistical significance. Table 3a shows the results of test of significance for every regression coefficient. The results showed that the p-value of the model terms were significant, i.e. $p < 0.05$. In this case, the four linear terms (X_1 , X_2 , X_3 , X_4), six cross-products (X_1X_2 , X_1X_3 , X_1X_4 , X_2X_3 , X_2X_4 , X_3X_4) and the four quadratic terms (X_{12} , X_{22} , X_{32} and X_{42}) were all remarkably significant model terms at 95% confidence level. In order to minimize error, all the coefficients were considered in the design. Table 3b shows the analysis of variance (ANOVA) of the regression equation. The model F-value of 317.10 implied a high significant for the regression model (Yuan et al., 2008). The goodness of the fit of a model was checked by the coefficient of determination (R^2). R^2 should be at least 0.80 for the good fit of a model (Guan and Yao, 2008). The obtainable R^2 of 0.9966 in this case indicated that the sample variation of 99.66% for AIME yield was attributed to the independent factors and only 0.34% of the total variations are not explained by the model.

Table 2: Central Composite Design, Experimental, Predicted and Residual Values for Five –Level-Four Factors Response Surface Analysis.

Std. Order	X ₁ (°C)	X ₂ (wt %)	X ₃ (min)	X ₄	Experimental value (w/w %)	Predicted value (w/w %)	Residual values (w/w%)
1	-1	-1	-1	-1	74.09	73.59	0.50
2	1	-1	-1	-1	73.5	73.28	0.22
3	-1	1	-1	-1	70.9	71.20	-0.30
4	1	1	-1	-1	68.00	68.53	-0.53
5	-1	-1	1	-1	84.88	85.32	-0.44
6	1	-1	1	-1	81.00	80.70	0.30
7	-1	1	1	-1	74.90	74.37	0.53
8	1	1	1	-1	68.00	67.40	0.60
9	-1	-1	-1	1	69.00	69.66	-0.66
10	1	-1	-1	1	64.5	65.14	-0.64
11	-1	1	-1	1	79.50	79.91	-0.41
12	1	1	-1	1	73.42	73.04	0.38
13	-1	-1	1	1	74.5	74.08	0.42
14	1	-1	1	1	65.5	65.26	0.24
15	-1	1	1	1	75.5	75.78	-0.28
16	1	1	1	1	64.00	64.61	-0.61
17	-2	0	0	0	75.50	75.26	0.24
18	2	0	0	0	63.71	63.77	-0.062
19	0	-2	0	0	63.26	63.31	-0.054
20	0	2	0	0	60.50	60.27	0.23
21	0	0	-2	0	87.5	86.86	0.64
22	0	0	2	0	89.69	90.15	-0.46
23	0	0	0	-2	73.50	74.02	-0.52
24	0	0	0	2	68.00	67.30	0.70
25	0	0	0	0	67.50	67.70	-0.20
26	0	0	0	0	68.00	67.70	0.30
27	0	0	0	0	67.50	67.70	-0.20
28	0	0	0	0	68.00	67.70	0.30
29	0	0	0	0	67.22	67.70	-0.48
30	0	0	0	0	68.00	67.70	0.30

Table 3a: ANOVA for Response Surface Quadratic Model Analysis of Variance Table

Source	Sum of Square	df	Mean Square	F-Value	Prob > F
X ₁	227.67	1	227.67	921.10	< 0.0001
X ₂	29.66	1	29.66	119.98	< 0.0001
X ₃	2.24	1	2.24	9.80	0.0087
X ₄	41.34	1	41.34	167.25	< 0.0001
X ₁ X ₂	4.37	1	4.37	17.67	0.0011
X ₁ X ₃	24.16	1	24.16	97.72	<0.0001
X ₁ X ₄	11.94	1	11.94	48.29	< 0.0001
X ₂ X ₃	95.84	1	95.84	387.72	< 0.0001
X ₂ X ₄	189.06	1	189.06	764.82	<0.0001
X ₃ X ₄	25.55	1	25.55	103.37	< 0.0001
X ₁ ²	13.76	1	13.76	55.67	<0.0001
X ₂ ²	33.06	1	33.06	133.75	< 0.0001
X ₃ ²	359.85	1	359.85	1455.71	< 0.0001
X ₄ ²	21.95	1	21.95	88.80	< 0.0001

Table 3b: Analysis of Variance (ANOVA) of Regression Equation

Source	Sum of squares	df	Mean Square	F-value	p-value
Model	1097.40	14	78.39	317.10	< 0.0001
Residual	3.71	15	0.25		
Lack of Fit	3.13	10	0.31	2.69	0.0740
Pure Error	0.58	5	0.12		
Cor Total	1101.11	29			
				R-Sq = 99.66%,	R-Sq(adj) = 99.35%

Table 4: ANOVA for Response Surface Quadratic Model for Intercept.

Factors	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	67.70	1	0.20	67.27	68.14	-
X ₁	-3.08	1	0.10	-3.30	-2.86	1.00
X ₂	-1.11	1	0.10	-1.33	-0.90	1.00
X ₃	0.31	1	0.10	0.09	0.52	1.00
X ₄	-1.31	1	0.10	-1.53	-1.10	1.00
X ₁ X ₂	-0.52	1	0.12	-0.79	-0.26	1.00
X ₁ X ₃	-1.23	1	0.12	-1.49	-0.96	1.00
X ₁ X ₄	-0.86	1	0.12	-1.13	-0.60	1.00
X ₂ X ₃	-2.45	1	0.12	-2.71	-2.18	1.00
X ₂ X ₄	3.44	1	0.12	3.17	3.70	1.00
X ₃ X ₄	-1.26	1	0.12	-1.53	-1.00	1.00
X ₁ ²	0.71	1	0.095	0.51	0.91	1.05
X ₂ ²	-1.10	1	0.095	-1.30	-0.90	1.05
X ₃ ²	3.62	1	0.095	3.42	3.82	1.05
X ₄ ²	0.89	1	0.095	0.69	1.10	1.05

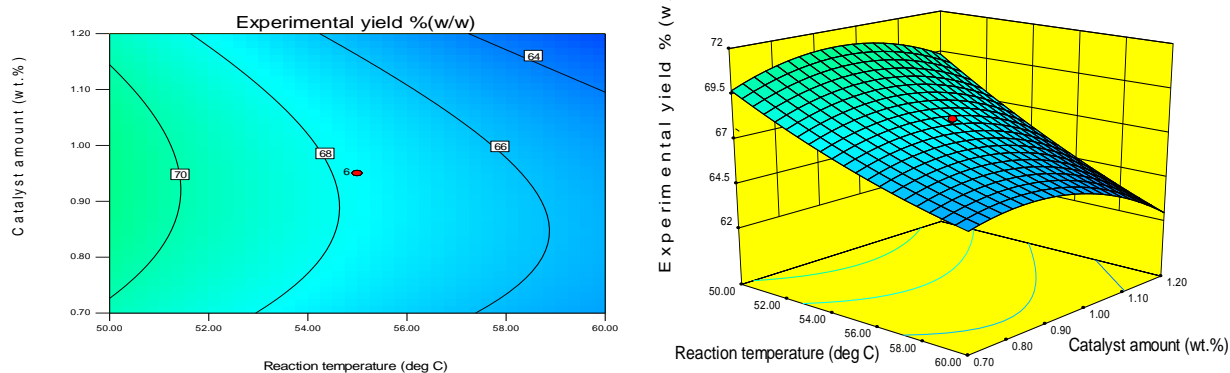
The value of adjusted determination coefficient ($\text{Adj. } R^2 = 0.9935$) was also very high, supporting a high significant of the model (Khuri and Cornell, 1987) and all p-value coefficients were less than 0.0001, which implied that the model proved suitable for the adequate representation of the actual relationship among the selected variables. The lack-of-fit term of 0.0740 was not

significant relative to the pure error. The final equation in terms of coded factors for the response surface quadratic model is expressed in Eq. (3).

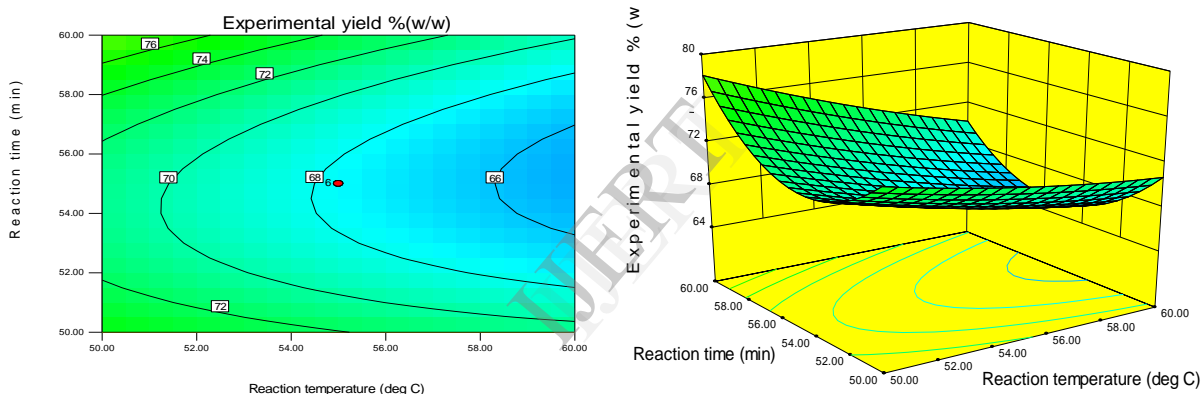
$$Y(w/w \%) = 67.70 - 3.08X_1 - 1.11X_2 + 0.31X_3 - 1.31X_4 - 0.52X_1X_2 - 1.23X_1X_3 - 0.86X_1X_4 - 2.45X_2X_3 + 3.44X_2X_4 - 1.26X_3X_4 + 0.71X_1^2 - 1.10X_2^2 + 3.62X_3^2 + 0.89X_4^2 \quad (3)$$

All the X_3 , X_1X_4 , X_{12} , X_{32} and X_{42} , (Table 4) had positive effect on the AIME yield while the rest had negative influence on the yield. In general, the 3D response surface plot is a graphical representation of the regression equation for the optimization of the reaction variables. Figure 1(a-f) described the 3D surfaces linked to the effect of two variables on the yield of AIME (biodiesel). Figure 1a revealed high yield of AIME at low reaction temperature and low catalyst amount. Whereas in Figure 1b, high reaction time and low reaction temperature favored AIME yield. Relationship between reaction temperature and methanol/oil molar ratio showed that low values of these factors supported high AIME yield (Figure 1c) while high reaction time and low catalyst amount gave high AIME yield (Figure 1d). Interaction between methanol/oil molar ratio and catalyst amount showed that low values of these two factors resulted in high yield of AIME. In the case of both reaction time and methanol/oil molar ratio, high reaction time and low methanol/oil molar ratio led to high AIME yield. The curvature nature of the 3D surfaces in Figure 1b, d and f indicated mutual interaction of reaction time with reaction temperature, reaction time with catalyst amount, and reaction time with methanol/oil molar ratio, respectively. The optimal condition predicted by the model were methanol/oil molar ratio 3:1, catalyst amount 0.70% (w/w), reaction temperature 50 °C, and reaction time 60 min, which gave 84.63% (w/w). Using these optimal condition values for three independent experimental

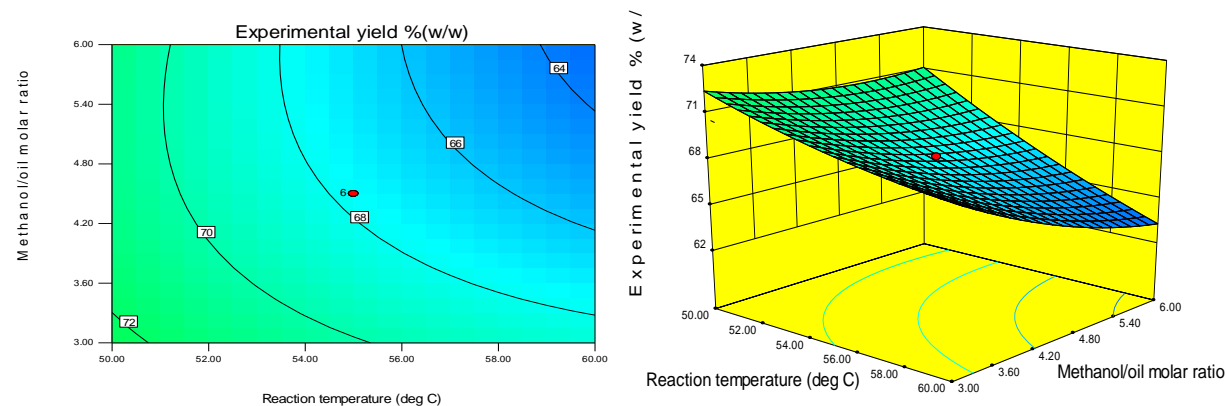
replicates, an average AIME yield of 85.13% (w/w) was achieved, which was within the range predicted by the model.



(a)

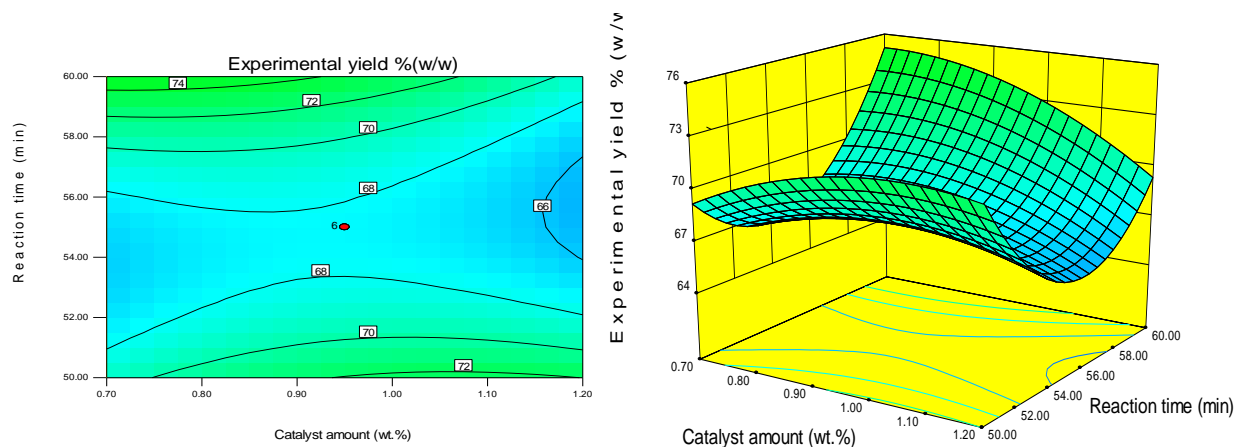


(b)

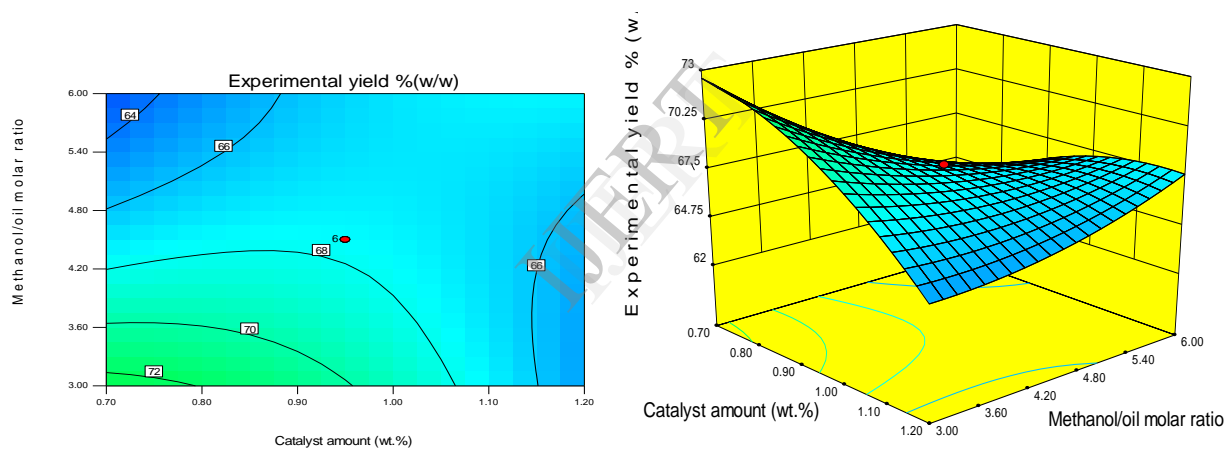


(c)

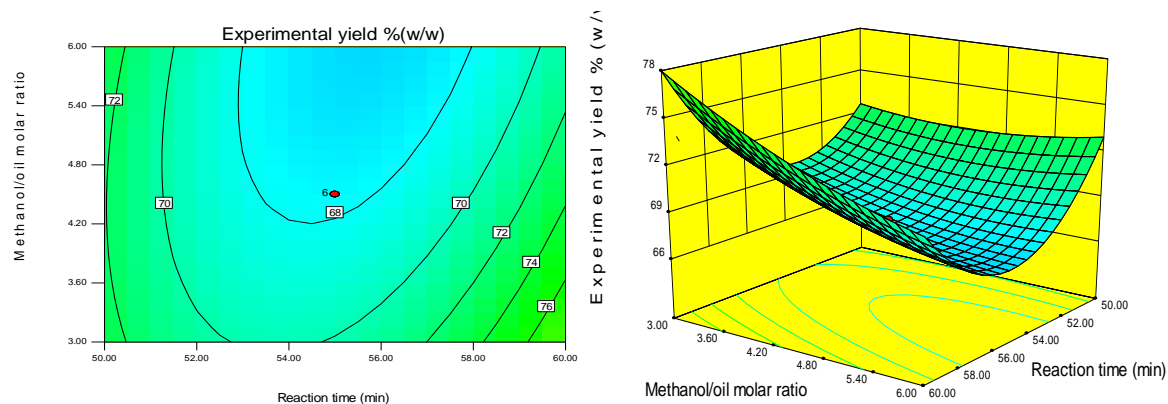
Figure 1(a-c): The contour and 3D plots of the effect of variables and their reciprocal interaction on experimental value keeping independent variables constant at zero level.



(d)



(e)



(f)

Figure 1(d-f): The contour and 3D plots of the effect of variables and their reciprocal interaction on experimental value keeping independent variables constant at zero level.

Quality and fuel properties of AIME

Table 5 shows the properties of the AIME in comparison with ASTM biodiesel and EN 14214 standards. All the tested characteristics and fuel properties of the AIME satisfied both the ASTM D 6751 and DIN EN 1424 standards. Gas chromatography analysis of fatty acids present in the AIME is shown in Table 6. The results indicated AIME was highly unsaturated. The dominant fatty acids were linoleic (61.28%), oleic (18.17%), stearic (9.15%) and palmitic (10.82%). The total unsaturated fatty acid composition of the AIME was 79.45%.

Table 5: Physicochemical and Other Characteristics of Methyl ester

Parameter	Methyl ester	ASTM	EN 14214
Physical state at 28°C	Liquid		
Moisture content (%)	0.05	0.05 max	-
Specific gravity	0.90	0.87-0.90	0.86-0.9
Kinematic viscosity (mm ² /s)	0.05	1.9-6.0	3.5-5.0
Saponification value (mg KOH/g oil)	207	-	-
Iodine value (g I ₂ /100g oil)	70.5	-	120 max
Higher heating value (MJ/kg)	39.89	-	-
Cetane number	54.38	-	-
Pour point (°C)	10	-15	-
Cloud point (°C)	-	6	12 max
Flash point (°C)	110	100 min	120 min

Table 6: Fatty Acids Compositions of the *Azadirachta indica* Oil and Methyl ester Produced

Parameters	Compositions %	
Palmitic acid (C16:0)	15.46	10.82
Palmitoleic acids (C16:1)	0.06	0.06
Stearic acids (C18:0)	0.22	9.15
Oleic acids (C18:1)	60.75	18.17
Linoleic acids (C18:2)	22.07	61.28
Linolenic acid (C18:3)	0.17	0.51
Arachidonic acid (C20:4)	1.05	0.00
Lignoceric Acid (C24:0)	0.03	0.00
Behenic acid (C22:0)	0.08	0.00
Other	0.11	0.01
Total	100	100

Emission characterization

The test was conducted on a four stroke, air cooled, single cylinder direct injection diesel engine, developing a power output of 3.23 kW at a constant of 2600 rpm. Table 7 shows the specifications of the engine. The characterization of fuel behavior with respect to emissions and performance was carried out by determined the CO and NO_x,

Table 7: Engine specifications

Parameter	Specification
Type of engine	Single cylinder
Engine brand name	165F, Direct injection, four-stroke, Internal Combustion Engine.
Stroke length	0.11 m
Bore and stroke	87.5 mm x 110 mm
Cooling method	Air
Injector operating pressure	200 bar/ 23 °C BTDC
Dynamometer current	Eddy current
Compression ratio	16.5:1

CO and NO emissions (ppm)

CO is only a very weak direct greenhouse gas, but has important indirect effects on global warming. CO is an ozone precursor, but to a lesser extent than unburned hydrocarbons or nitrogen oxides. Biomass burning and fossil fuel use are the main sources of man-made CO emission. The most potential control is through direct reduction in fossil fuel use. Since the emission of CO depended on rotational speed, it decreased with increased in concentration of biodiesel.

Likewise, NO_x should not be confused with N₂O, which is a greenhouse gas. It is the total concentration of NO and NO₂. When NO_x and volatile organic compounds (VOCs) react in the presence of sunlight, they form photochemical smog, a significant form of air pollution, especially in the summer. Its adverse effect is damage to the lung tissue and reduction in lung function [22]. It can also form nitric acid which contributes to acid rain if the combustion

emission is not regulated in the environment. NO_x also increase in proportion to ignition advance, regardless of variations in the air/fuel ratio.

Hence, the CO and NO emission concentration of blends decrease by 45% and 40%, (Figure 3-4) respectively, compared to the conventional diesel fuel thus, help reduction in emissions that have harmful effect on the environment.

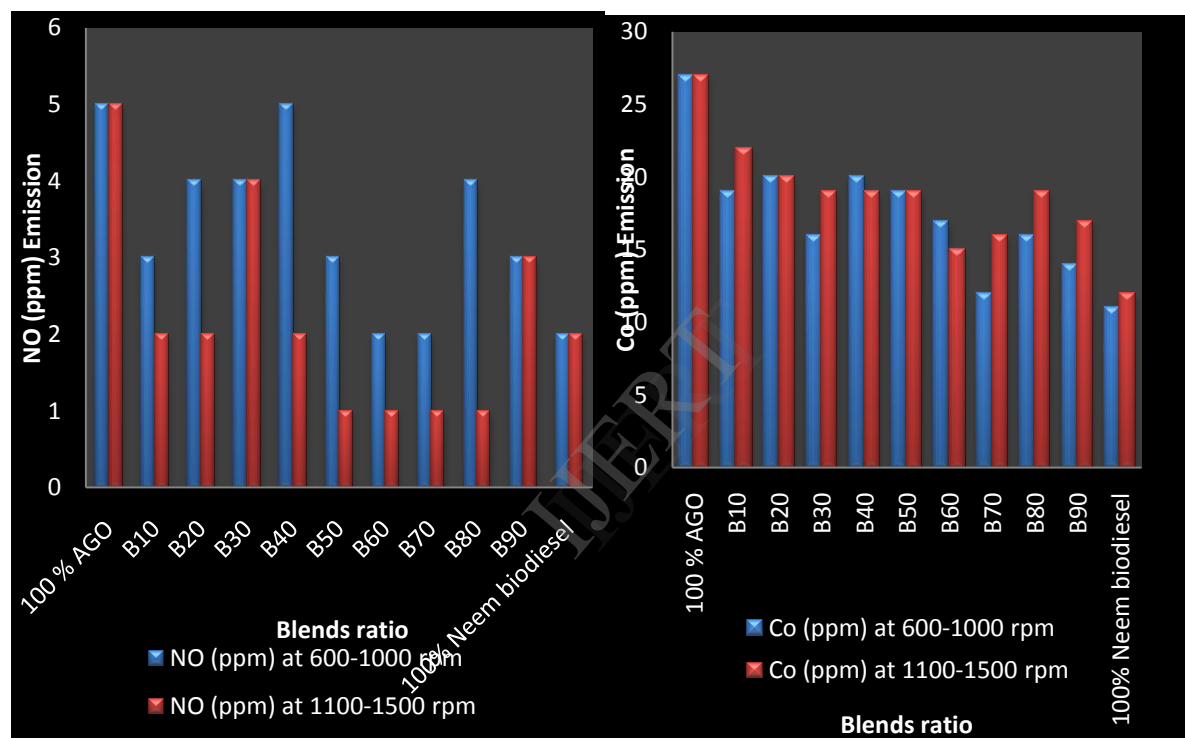


Figure 3-4: Fuel Stability and Emissions from Combustion of Neem Biodiesel Plots

Conclusions

In this study, experiments were conducted using RSM to determine the effects of four reaction factors namely methanol/oil molar ratio, reaction temperature, catalyst concentration and reaction time on AIME yield in the transesterification of the neem seed oil. The maximum AIME conversion yield was validated as 85.13% (w/w) under the optimal reaction condition of 3:1 methanol/oil molar ratio, 50 °C reaction temperature, 0.70% catalyst concentration, and reaction

time of 60 min. The fuel properties of AIME satisfied both the ASTM D 6751 and DIN EN 1424 standards. Thus, the present study demonstrates the usefulness of RSM for optimum conversion of *Azadirachta indica* seed oil to AIME. It also suggests that the seed oil could be used effectively as feedstock for AIME production. The CO and NO emission concentration of blends decrease by 45% and 40%, respectively, compared to the conventional diesel fuel thus, help reduction in emissions that have harmful effect on the environment.

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